

Attorney Docket No. 3477-94
In re: Gawad
Serial No.: 10/018,614
Filed: April 15, 2002
Page 2

Listing of Claims

The following list of claims will replace all prior versions and listings of claims in the application.

1. (Currently Amended) A method for conducting a binding assay to detect the presence of an analyte in a solution, comprising the steps of:
 - (a) contacting a first binding partner of the analyte with said solution, said first binding partner being conjugated to a calcium-sensitive chemiluminescent material;
 - (b) after a period of time, mobilizing the first binding partner in a predetermined direction along one side of ~~an elongated matrix of a capture strip comprising an immobilized, second binding partner of the analyte and a calcium-containing calcium-caging compound, so as to~~ contact the first binding partner with ~~a stripe transversely located on said capture strip, said transverse stripe comprising an~~ said immobilized second binding partner of the analyte and containing a calcium-containing calcium-caging compound;
 - (c) allowing a period of time sufficient for the first binding partner to contact said second binding partner immobilized on said ~~transverse stripe, capture strip;~~
 - (d) exposing said ~~transverse stripe of said capture strip~~ to a pulse of ultraviolet light to effect the release of calcium from the calcium-caging compound; ~~and~~
 - (e) resetting a photomultiplier to its zero or null point after said pulse; and
 - (f) measuring luminescence emitted by the calcium-sensitive luminescent material with said photomultiplier,wherein the calcium-sensitive chemiluminescent material ~~and the~~

Attorney Docket No. 3477-94
In re: Gawad
Serial No.: 10/018,614
Filed: April 15, 2002
Page 3

~~calcium-caging compound are~~ is selected so that there is a period with no light emission between the pulse of ultraviolet light effecting calcium release and the emission of luminescence by the luminescent material of sufficient duration to allow the resetting of the photomultiplier.

2. (Currently Amended) ~~The A method of Claim 1~~ for conducting a binding assay to detect the presence of an analyte in a solution, comprising the steps of:

(a) contacting said solution with a first binding partner of the analyte, said first binding partner being immobilized on a solid surface, said solid surface being paramagnetic particles and said first binding partner being conjugated to a calcium-sensitive luminescent material;

(b) after a period of time, mobilizing the paramagnetic particles in a predetermined direction along one side of ~~an elongated matrix of a capture strip comprising an immobilized, second binding partner of the analyte and a calcium-containing calcium-caging compound so as to~~ contact the particles with ~~the a stripe of a second binding partner of the analyte transversely located on said capture strip, said capture strip having the second binding partner immobilized on said transverse stripe, said transverse stripe additionally containing a calcium-caging compound,;~~

(c) allowing a period of time sufficient for the paramagnetic particles to contact said second binding partner immobilized onto said transverse stripe,

(d) exposing said transverse stripe of said capture strip to a pulse of ultraviolet light to effect release of calcium from the calcium caging compound; ~~and~~

(e) resetting a photomultiplier to its zero or null point after said pulse; and

Attorney Docket No. 3477-94
In re: Gawad
Serial No.: 10/018,614
Filed: April 15, 2002
Page 4

(f) measuring luminescence emitted by the calcium-sensitive luminescent material with said photomultiplier.

wherein the calcium-sensitive luminescent material is selected so that there is a period with no light emission between the pulse of ultraviolet light effecting calcium release and the emission of luminescence by the luminescent material of sufficient duration to allow the resetting of the photomultiplier.

3. (Original) The method of Claim 2 in which the method is an immunoassay for detecting and quantifying an antigen, an immunoassay for detecting and quantifying an antibody, or a nucleic acid hybridization assay for detection and quantifying a particular sequence of nucleic acid.

4. (Original) The method of Claim 1 in which the solution is pretreated prior to contacting the calcium sensitive luminescent material in step (a).

5. (Currently Amended) The method of Claim 4 in which the pretreatment comprises a filtration step that removes calcium~~solution is passed through a filter to remove calcium, the filter being impregnated with an agent for removal of calcium.~~

6. (Original) The method of Claim 1 in which the solution is whole blood, said whole blood being pretreated by filtering prior to being contacted with the calcium sensitive luminescent material.

7. (Original) The method of Claim 1 in which the calcium-sensitive luminescent material is aequorin, Obelin, Mnemiopsin, Berovin, Pholasin, Luciferases or photoproteins isolated from Pelagia, Cypridina and

Attorney Docket No. 3477-94
In re: Gawad
Serial No.: 10/018,614
Filed: April 15, 2002
Page 5

ostracods.

8. (Currently Amended) The method of Claim 1 in which the ultraviolet light is in the form of a pulse of light in the range of 250-400 nm, ~~and the luminescence is measured by a photomultiplier.~~

9. (Currently Amended) The method of Claim ~~8~~1 in which the calcium-sensitive luminescent material is aequorin and in which the photomultiplier detects light of 400-600 nm and is protected from the magnetic field.

10. (Currently Amended) The method of Claim 1 in which the ~~elongated~~ capture strip is formed of nitrocellulose, polyacrylamide or any other natural or synthetic polymer.

11. (Currently Amended) The method of Claim ~~10~~1 in which the ~~elongated~~ capture strip has a transverse stripe containing the with immobilized second binding partner and ~~the impregnated with a calcium-caging compound.~~

12. (Original) The method of Claim 1 in which the calcium caging compound is loaded with calcium in excess of the stoichiometric amount for said calcium-sensitive luminescent material.

13. (Original) The method of Claim 1 in which the calcium-caging compound is selected from the group consisting of cis-1-(2-bis(carboxymethyl)amino-5-(1-hydroxy-1-(2-nitro-4,5-methylenedioxyphenyl)methyl)phenoxy)-2-(2-bis(carboxymethyl)amino-

Attorney Docket No. 3477-94

In re: Gawad

Serial No.: 10/018,614

Filed: April 15, 2002

Page 6

5-methylphenoxy)cyclopentane, 1-[2-Amino-5-(1-hydroxy-1-[2-nitro-4,5-methylenedioxyphenyl]methyl)phenoxy]-2-)2'-amino-5'-methylphenoxy)ethane-N,N,N',N'-tetraacetic acid, 1-(4,5 dimethoxy-2-nitrophenyl)-1,2 diaminoethane-N,N,N', N'-tetraacetic acid and nitrophenyl-ethylenebis(oxyethylenenitrilo)tetraacetic acid.

14. (Currently Amended) The method of Claim 1 in which the binding assay is an immunosassay for detecting and quantifying an antigen.

15. (Currently Amended) The method of Claim 1 in which the binding assay is an immunoassay for detecting and quantifying an antibody.

16. (Original) The method of Claim 1 in which the binding assay is nucleic acid hybridization assay for detection and quantifying a particular sequence of nucleic acid.

17. (Original) The method of Claim 1 in which the calcium-sensitive luminescent marterial is aequorin.

18. (Original) The method of Claim 1 in which the ultraviolet light source emits a pulse of light in the range of 250-400 nm.

19. (Cancelled)

20. (Original) The method of Claim 1 in which the calcium-sensitive luminescent material is aequorin and photomultiplier detects light of 400-600 nm and is protected from the magnetic field.

Attorney Docket No. 3477-94
In re: Gawad
Serial No.: 10/018,614
Filed: April 15, 2002
Page 7

21. (Currently Amended) A method for conducting a binding assay to detect the presence of an analyte in a solution, comprising the steps of:

(a) immobilizing a first binding partner of the analyte on a solid surface, said solid surface being paramagnetic particles, said first binding partner being biotinylated;

(b) contacting said first binding partner with said solution;

(c) contacting the solution with a second binding partner of the analyte, said second binding partner being conjugated to a calcium-sensitive luminescent material;

(d) after a period of time, mobilizing the paramagnetic particles in a predetermined direction along one side of ~~an elongated matrix of a capture strip, so as to contact the particles with a stripe transversely located on said capture strip,~~ said capture strip comprising immobilized having streptavidin immobilized onto said transverse stripe, said transverse stripe additionally contain and a calcium-caging compound,

(e) allowing a period of time sufficient for the paramagnetic particles to contact said immobilized streptavidin; ~~immobilized onto said transverse stripe,~~

(f) exposing said ~~transverse stripe of said capture strip~~ to a pulse of ultraviolet light to effect the release of calcium from the calcium caging compound; and

(g) resetting a photomultiplier to its zero or null point after said pulse; and

(h) measuring luminescence emitted by the calcium-sensitive luminescent material with said photomultiplier,

wherein the calcium-sensitive chemiluminescent material ~~and the calcium-caging compound~~ are ~~is~~ selected so that there is a period with no light emission between the pulse of ultraviolet light effecting calcium

Attorney Docket No. 3477-94
In re: Gawad
Serial No.: 10/018,614
Filed: April 15, 2002
Page 8

release and the emission of luminescence by the luminescent material of sufficient duration to allow the resetting of the photomultiplier.

22. (Original) The method of Claim 21 in which steps (b) and (c) are carried out simultaneously.

23. (Currently Amended) A method for conducting a binding assay to detect the presence of an analyte in a solution, comprising the steps of:

- (a) contacting a first binding partner of the analyte with said solution, said first binding partner being biotinylated;
- (b) after a period of time, contacting the solution with a second binding partner of the analyte, said second binding partner being conjugated to a calcium-sensitive luminescent material;
- (c) after a further period of time, mobilizing the first and second binding partners in a predetermined direction along one side ~~of an elongated matrix~~ of a capture strip so as to contact the binding partners with a stripe transversely located on said capture strip, said capture strip comprising immobilized having streptavidin immobilized onto said transverse stripe, said transverse stripe additionally contain and a calcium-caging compound;
- (d) allowing a period of time sufficient for the binding partners to contact said streptavidin immobilized onto said transverse stripe,
- (e) exposing said transverse stripe of said capture strip to a pulse of ultraviolet light to effect the release of calcium from the calcium caging compound; and
- (f) resetting a photomultiplier to its zero or null point after said pulse; and
- (g) measuring luminescence emitted by the calcium-sensitive

Attorney Docket No. 3477-94
In re: Gawad
Serial No.: 10/018,614
Filed: April 15, 2002
Page 9

luminescent material with said photomultiplier,

wherein the calcium-sensitive chemiluminescent material is and the
~~calcium-caging compound~~ are selected so that there is a period with no
light emission between the pulse of ultraviolet light effecting calcium
release and the emission of luminescence by the luminescent material of
sufficient duration to allow the resetting of the photomultiplier.

24. (Original) The method of Claim 23 in which steps (a) and (b) are
carried out simultaneously.

25. (Currently Amended) The method of Claim 21 in which the ~~elongated~~
~~capturing~~ capture strip has a transverse ~~section thereof impregnated with~~
stripe containing the immobilized streptavidin and the a-calcium-caging
compound.

26. (Cancelled)

27. (Original) The method of Claim 1 in which the free calcium
concentration of the solution is less than 20 nanomolar before the pulse of
ultraviolet light.

28. (Withdrawn) An elongated capture strip for binding assays, said strip
having a transverse section thereof impregnated with streptavidin and a
calcium caging compound.

29. (Withdrawn) The elongated capture strip of Claim 28 in which the
capture strip is formed from nitrocellulose, polyacrylamide, polyamide or
any other synthetic or naturally occurring polymer.

Attorney Docket No. 3477-94

In re: Gawad

Serial No.: 10/018,614

Filed: April 15, 2002

Page 10

30. (Withdrawn) The elongated capture strip of Claim 28 in which the capture strip is in a housing.

31. (Withdrawn) The elongated capture strip of Claim 30 in which the capture strip is housed within a support as a single use testing cartridge.

32. (Withdrawn) The elongated capture strip of Claim 28 in which calcium-caging compound is selected from the group consisting of cis-1-(2-bis(carboxymethyl)amino-5-(1-hydroxy-1-(2-nitro-4,5-methylenedioxyphenyl)methyl)phenoxy)-2-(2-bis(carboxymethyl)amino-5-methylphenoxy)cyclopentane, 1-[2-Amino-5-(1-hydroxy-1-[2-nitro-4,5-methylenedioxyphenyl]methyl)phenoxy]-2-)-2'-amino-5'-methylphenoxy)ethane-N,N,N',N'-tetraacetic acid, 1-(4,5 dimethoxy-2-nitrophenyl)-1,2 diaminoethane-N,N,N',N'-tetraacetic acid and nitrophenyl-ethylenebis(oxyethylenetrinitrilo)tetraacetic acid.

33. (Withdrawn) A plastic cartridge for conducting a binding assay to detect the presence of analyte in a solution, comprising:

a housing with a receptacle for receipt of a sample, a reservoir containing biotinylated first binding partner immobilized onto paramagnetic particles and a second binding partner conjugated to calcium-sensitive chemiluminescence material, an elongated capture strip within the housing and in fluid communication with the reservoir, said capture strip having a transverse section thereof impregnated with a calcium-caging compound and streptavidin, said transverse section being protected with a light barrier.

Attorney Docket No. 3477-94
In re: Gawad
Serial No.: 10/018,614
Filed: April 15, 2002
Page 11

34. (Withdrawn) The plastic cartridge of Claim 33 in which there is a filter between the receptacle and the reservoir.
35. (Withdrawn) The plastic cartridge of Claim 33 in which there is a filter containing an agent for removal of calcium.
36. (Withdrawn) The plastic cartridge of Claim 33 in which the calcium-sensitive luminescent material is aequorin, Obeln, Mnemiopsin, Berovin, Pholasin, Luciferases or photoproteins isolated from Pelagia, Cypridina and ostracods.
37. (Withdrawn) Apparatus for carrying out a binding assay comprising a housing enclosing (a) a receptacle to receive the plastic cartridge of Claim 33; (b) a means for removing the light protective layer over the transverse stripe; (c) an electromagnet to provide a magnetic field; (e) an ultraviolet light source to project light on a pre-selected portion of the capture strip, and (f) a photomultiplier disposed to receive light emitted by the pre-selected portion of the capture strip.
38. (Withdrawn) The apparatus of Claim 37 in which the electromagnet projects multiple magnetic fields along the plastic cartridge.
39. (Withdrawn) The apparatus of Claim 37 in which the ultraviolet light source provides light in the range of 250-400 nm.
40. (Withdrawn) The apparatus of Claim 37 in which the photomultiplier detects light in the range of 400-600 nm.

Attorney Docket No. 3477-94

In re: Gawad

Serial No.: 10/018,614

Filed: April 15, 2002

Page 12

41. (Currently Amended) The method of eClaim 1 wherein the calcium-caging compound is loaded with up to 75% calcium.
42. (Original) The method of claim 21 wherein the calcium-caging compound is loaded with up to 75% calcium.
43. (Original) The method of claim 23 wherein the calcium-caging compound is loaded with up to 75% calcium.
44. (Currently Amended) The method of Celaim 21 in which the free calcium concentration of the solution is less than 20 nanomolar of calcium before the pulse of ultraviolet light.
45. (Original) The method of claim 23 in which the free calcium concentration of the solution is less than 20 nanomolar of calcium before the pulse of ultraviolet light.
46. (Original) The method of claim 27 wherein the calcium-caging compound is loaded with up to 75% calcium.
47. (Currently Amended) The method of Celaim 44 wherein the calcium-caging compound is loaded with up to 75% calcium.
48. (Original) The method of claim 45 wherein the calcium-caging compound is loaded with up to 75% calcium.
49. (Previously Presented) The method of Celaim 17 wherein the calcium-caging compound is DM-nitrophen.

Attorney Docket No. 3477-94

In re: Gawad

Serial No.: 10/018,614

Filed: April 15, 2002

Page 13

50. (Currently Amended) The method of Claim 21 wherein the calcium-sensitive luminescent material is Aequorin and the calcium-caging compound is DM-nitrophen.

51. (Original) The method of claim 23 wherein the calcium-sensitive luminescent material is Aequorin and the calcium-caging compound is DM-nitrophen.

52. (Currently Amended) The method of Claim 1 wherein the calcium-sensitive luminescent material is Obelin and the calcium-caging compound is DM-nitrophen.

53. (Original) The method of claim 21 wherein the calcium-sensitive luminescent material is Obelin and the calcium-caging compound is DM-nitrophen.

54. (Original) The method of claim 23 wherein the calcium-sensitive luminescent material is Obelin and the calcium-caging compound is DM-nitrophen.